ORIGINAL INVESTIGATION

Relationship Between Systemic Markers of Inflammation and Serum β-Carotene Levels

Thomas P. Erlinger, MD, MPH; Eliseo Guallar, MD, DrPH; Edgar R. Miller III, MD, PhD; Rachael Stolzenberg-Solomon, PhD, RD; Lawrence J. Appel, MD, MPH

Background: Low serum levels of β-carotene have been associated with increased risk of cancer and cardiovascular disease. However, in clinical trials, supplementation of the diet with β-carotene either had no benefit or caused harm. This pattern of findings raises the possibility that confounding by other factors might explain the association between serum β-carotene level and disease risk.

Methods: We used data from 14 470 current smokers, ex-smokers, and never smokers aged 18 years or older who participated in the Third National Health and Nutrition Examination Survey to assess the relationship between serum β-carotene and markers of inflammation (C-reactive protein and white blood cell count).

Results: After adjustment for β-carotene intake and other factors, geometric mean levels of serum β-carotene for individuals with undetectable (<0.22 mg/dL), mildly elevated (0.22-0.99 mg/dL), and clinically elevated (≥1.0 mg/dL) C-reactive protein levels were 18.0, 16.1, and 13.6 μg/dL, respectively, in never smokers; 18.1, 15.7, and 13.9 μg/dL in ex-smokers; and 11.3, 10.2, and 9.4 μg/dL in current smokers ($P<.001$ for all). In corresponding analyses, white blood cell count was also inversely related to serum β-carotene concentration ($P<.05$ for all).

Conclusions: The strong and inverse association of serum β-carotene level with C-reactive protein level and white blood cell count suggests that the relationship between serum β-carotene concentration and disease risk might be confounded by inflammation. More broadly, for β-carotene and likely other nutrients, it seems unwise to interpret biomarker data as prima facie evidence of dietary intake without a more complete understanding of the physiologic processes that affect nutrient levels.

Arch Intern Med. 2001;161:1903-1908

From the Welch Center for Prevention, Epidemiology, and Clinical Research (Drs Erlinger, Guallar, Miller, and Appel) and the Departments of Medicine (Drs Erlinger, Miller, and Appel) and Epidemiology (Drs Guallar and Appel), Johns Hopkins Medical Institutions, Baltimore, Md; and the National Cancer Institute, National Institutes of Health, Bethesda, Md (Dr Stolzenberg-Solomon).

In a landmark article, Peto et al[1] hypothesized that low intake of β-carotene was a modifiable risk factor for cancer. This hypothesis was strongly supported by the consistent finding of an inverse association between serum β-carotene level and risk of cardiovascular disease and certain types of cancer, especially lung cancer.[2,3] However, in large-scale trials,[4-10] supplementation of the diet with β-carotene either had no benefit or caused harm. There are several possible explanations for the discrepancy between results of observational studies and clinical trials,[2] including the possibility of confounding by other nutrients or lifestyle factors that might be associated with β-carotene intake. An alternative explanation is that serum β-carotene levels reflect not only β-carotene intake but also other physiologic processes related to disease occurrence. In that case, low serum β-carotene concentration might be an epiphenomenon, and increased intake of β-carotene would not be expected to reduce the risk of disease. Although this is a well-known theoretical limitation of serum biomarkers, it is seldom considered in the interpretation of biomarker data.

Preliminary evidence suggests that β-carotene levels are associated with inflammation. For example, it is well known that smoking increases systemic markers of inflammation[11] and that smokers have lower levels of serum β-carotene than nonsmokers independent of β-carotene intake.[12,13] In elderly women and in persons with lung cancer, an inverse relationship between inflammatory markers and serum β-carotene concentration has been documented.[14,15] In middle-aged adults, serum level of sialic acid, a systemic marker of inflammation, was inversely associated with serum β-carotene level.[16] Inverse associations between serum β-carotene level and C-reactive protein (CRP) level, an acute-phase reactant, have also been found in persons who are critically ill[17] or have other acute in-

©2001 American Medical Association. All rights reserved.

PARTICIPANTS AND METHODS

STUDY POPULATION

The NHANES III is a national probability survey of Americans conducted between 1988 and 1994 by the National Center for Health Statistics of the Centers for Disease Control and Prevention. This survey used a complex, multistage, stratified, cluster-sampling design to obtain a representative sample of the noninstitutionalized civilian US population. Of the 19618 NHANES III participants aged 18 years and older, we excluded 2989 with missing serum β-carotene data, 1943 with unrealistic total caloric intake (<800 or >4200 kcal/d in men and <600 or >3500 kcal/d in women), and 216 who were pregnant, leaving 14470 individuals available for analysis.

MEASUREMENTS

A detailed description of survey methods and data collection procedures has been published elsewhere.[28,29] In brief, questionnaire data included self-reported age, race or ethnicity, sex, and medical history. Nutrient intake was estimated from a single 24-hour dietary recall. Nonfasting blood samples were used for analysis of inflammatory markers, total cholesterol level, and β-carotene concentration.

Serum β-carotene level was measured using high-performance liquid chromatography. The interbatch coefficient of variation of pooled samples used for quality control varied between 5.7% and 10.0%. This assay could detect a serum β-carotene level of 0.67 µg/dL or greater. Only 5 participants had serum β-carotene levels below the limit of detection. The CRP level was measured using latex-enhanced nephelometry. Pooled controls had a coefficient of variation of 3.2% to 16.1% through the period of data collection. Because 74% of individuals had CRP levels below the detection limit for this assay (0.22 mg/dL), we treated CRP level as a categorical variable: undetectable (<0.22 mg/dL), mildly elevated (0.22-0.99 mg/dL), and clinically elevated (≥1.00 mg/dL). The WBC count was determined using a fully automated hematology analyzer (Counter Model S-PLUS JR; Coulter Electronics, Hialeah, Fla). Serum cholesterol level was measured enzymatically (Hitachi 704 Analyzer; Boehringer Mannheim Diagnostics, Indianapolis, Ind).

Participants were classified as never smokers, ex-smokers, and current smokers. Never smokers and ex-smokers were defined by self-report, whereas current smokers were defined by self-report or by a serum cotinine level greater than 57 nmol/L, as measured by high-performance liquid chromatography and atmospheric-pressure chemical ionization tandem mass spectroscopy. Diabetes mellitus was defined by self-report of a physician diagnosis, by the presence of a fasting plasma glucose level greater than 126 mg/dL (>7.0 mmol/L), or by the presence of a 2-hour glucose tolerance test result greater than 200 mg/dL (>11.1 mmol/L). Prevalent cardiovascular disease was defined by self-report of physician-diagnosed myocardial infarction or stroke or by angina as assessed by the Rose questionnaire. Information on current use of estrogen replacement therapy, use of vitamin or mineral supplements in the past month, and use of aspirin or other nonsteroidal anti-inflammatory drugs during the past month was based on self-report.

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured at the level of the high point of the iliac crest and hip circumference at the level of maximum extension of the buttocks. The waist-hip ratio (WHR) was calculated as waist circumference divided by hip circumference. Blood pressure measurement was the average of measurements obtained at the household interview and the mobile examination center (maximum of 3 measurements at each).

STATISTICAL METHODS

Because the distribution of serum β-carotene levels was right-skewed, we log-transformed this variable and then back-transformed the results for this study. The association between serum β-carotene concentration and participant characteristics was evaluated by quintiles of serum β-carotene level using multiple linear regression for continuous outcomes and logistic regression for dichotomous outcomes. Multiple linear regression was used to determine whether serum β-carotene level was independently associated with markers of inflammation. In addition to age, race, and sex, variables in this model included known determinants of serum β-carotene levels (total caloric intake, dietary fat and carotenoid intake, serum cholesterol level, BMI, WHR, and use of vitamin or mineral supplements) and factors or conditions associated with systemic markers of inflammation (estrogen replacement therapy, aspirin, or other nonsteroidal anti-inflammatory drug use; diabetes mellitus; and prevalent cardiovascular disease). These variables were selected a priori, before introducing systemic markers of inflammation into the models. Because of the well-known association of smoking with low levels of serum β-carotene and systemic markers of inflammation, all analyses were stratified by smoking status (never smokers, ex-smokers, and current smokers).[11,25,30] Tests for trend were performed by adding a continuous variable with the median of each category into the regression models.

To account for the complex survey design and to obtain results generalizable to the US noninstitutionalized population, we used SUDAAN software[31] and applied NHANES III weights in all analyses. $P<.05$ was considered statistically significant (2-sided).

flammatory conditions, such as pancreatitits and tuberculosis.[18,19] However, interpretation of these study results is complicated by the lack of control of important confounders, including smoking status and dietary intake of β-carotene.

The importance of these findings is highlighted by the fact that systemic markers of inflammation are consistently associated with arteriosclerotic cardiovascular disease (ASCVD) events and tumor recurrence.[20,21] Numerous studies[22-27] have now shown that CRP level and white blood cell (WBC) count independently predict ASCVD events. In this setting, we hypothesized that serum β-carotene level is inversely associated with systemic markers of inflammation in the general population and that this association is independent of smoking status, dietary intake of β-carotene, and other potential confounders. To test this

©2001 American Medical Association. All rights reserved.

hypothesis, we used data from the nationally representative cohort of the Third National Health and Nutrition Examination Survey (NHANES III).

RESULTS

Table 1 displays characteristics of the 14470 participants included in our analyses. On average, ex-smokers were older and were more likely to be white than never or current smokers. Ex-smokers also had higher blood pressure, cholesterol levels, BMI, and WHR, as well as a higher prevalence of diabetes mellitus and ASCVD. Current smokers had higher CRP levels and WBC counts and lower levels of serum β-carotene than ex-smokers or never smokers.

In the 3 smoking categories, individuals with higher levels of serum β-carotene tended to be older and were more likely to be female and white than those with lower serum β-carotene levels (**Table 2**). After adjusting for age, sex, and race, serum β-carotene level was positively associated with total serum cholesterol level, carotenoid intake, and use of vitamin or mineral supplements during the past month and inversely associated with BMI and WHR in the 3 smoking categories. Total caloric intake was positively associated with serum β-carotene level, but the trend reached statistical significance in never smokers only ($P<.001$). Fat intake was inversely associated with serum β-carotene level in never smokers and ex-smokers but positively associated in current smokers. Finally, an inverse association between alcohol intake and serum β-carotene level was present only in current smokers.

ASSOCIATIONS BETWEEN β-CAROTENE AND CRP LEVELS

The level of CRP was strongly and inversely related to serum level of β-carotene. After adjusting for age, sex, and race (**Table 3**, model 1), the geometric mean levels of serum β-carotene in never-smokers with undetectable, mildly elevated, and clinically elevated CRP levels were 18.9, 14.7, and 11.0 µg/dL, respectively ($P<.001$ for trend). After further adjustment for serum cholesterol level, BMI, WHR, total caloric intake, alcohol intake, use of vitamin or mineral supplements, systolic blood pressure, use of aspirin or nonsteroidal anti-inflammatory drugs, estrogen replacement therapy, diabetes mellitus, and prevalent cardiovascular disease (Table 3, model 2), the relationship between β-carotene and CRP levels persisted. The corresponding geometric means were 18.0, 16.1, and 13.6 µg/dL, respectively ($P<.001$ for trend).

The association between CRP and serum β-carotene levels in ex-smokers was similar to that of never smokers. In multivariate analysis (Table 3, model 2), geometric mean levels of serum β-carotene in ex-smokers with undetectable, mildly elevated, and clinically elevated CRP were 18.1, 15.7, and 13.9 µg/dL, respectively ($P<.001$ for trend). Current smokers had markedly lower levels of serum β-carotene, but an inverse association with CRP level was still evident. In multivariate analysis (Table 3, model 2), serum β-carotene levels in smokers with undetectable, mildly elevated, and clinically elevated CRP levels were 11.6, 11.7, and 8.3 µg/dL, respectively ($P<.001$ for trend).

Table 1. Characteristics of 14 470 Adults Who Participated in the Third National Health and Nutrition Examination Survey According to Smoking Status*

	Never Smokers (n = 6973)	Ex-smokers (n = 3105)	Current Smokers (n = 4392)
Age, mean, y	42.9	51.8	40.9
Sex, % female	64	44	44
Race or ethnicity, %			
Non-Hispanic white	72	84	78
Non-Hispanic black	11	6	12
Mexican American	6	4	4
Other	11	6	6
Systolic blood pressure, mean, mm Hg	121.3	126.5	120.5
Diastolic blood pressure, mean, mm Hg	73.7	75.5	73.6
Serum cholesterol, mean, mg/dL†	202	211	201
Body mass index, mean, kg/m^2	26.4	27.5	25.9
Waist-hip ratio, mean	0.89	0.93	0.91
Total calorie intake, mean, kcal/d	1985.9	2051.8	2160.8
Fat intake, mean, % kcal	32.9	33.3	34.2
Carotenoid intake, mean, RE/1000 kcal/d	318.1	317.3	197.5
Alcohol intake, % current drinkers	17	27	30
Multivitamin or mineral supplement use during the past month, %	45	48	34
Aspirin or NSAID use during the past month, %	52	56	56
Estrogen replacement therapy, % of women	7	12	7
Diabetes mellitus, %	9	16	8
Prevalent ASCVD, %	5	11	7
Serum β-carotene, µg/dL‡	17.4	17.1	11
White blood cell count, mean, /µL	6770	6952	8037
C-reactive protein, %			
<0.22 mg/dL	74	70	70
0.22-0.99 mg/dL	20	22	21
≥1.00 mg/dL	6	8	9

*RE indicates retinol equivalents; NSAID, nonsteroidal anti-inflammatory drug; and ASCVD, arteriosclerotic cardiovascular disease.

†To convert cholesterol from milligrams per deciliter to millimoles per liter, multiply milligrams per deciliter by 0.02586.

‡Geometric means. The medians (25th and 75th percentiles) β-carotene values were 16.5 µg/dL (9.9-27.3 µg/dL) in never smokers, 16.8 µg/dL (9.7-27.3 µg/dL) ex-smokers, and 10.1 µg/dL (6.3-16.7 µg/dL) in current smokers.

ASSOCIATION BETWEEN β-CAROTENE LEVEL AND WBC COUNT

A strong inverse association was also present between WBC count and serum β-carotene level (**Table 4**). Af-

©2001 American Medical Association. All rights reserved.

Table 2. Factors Associated With Serum β-Carotene Level According to Smoking Status*

	Quintile of Serum β-Carotene†								
	Never Smokers			Ex-smokers			Current Smokers		
	Lowest	Highest	P‡	Lowest	Highest	P‡	Lowest	Highest	P‡
Age, y	34.3	50.8	<.001	47.5	55.8	<.001	37.2	48.9	<.001
Sex, % women	52	73	<.001	29	54	<.001	30	56	<.001
Race, % white	64	75	.02	82	86	.08	74	78	.14
Serum cholesterol, mg/dL§	195.9	208.9	<.001	199.5	211.1	.009	188.7	213.3	<.001
Body mass index, kg/m^2§	29.1	24.4	<.001	29.7	25.1	<.001	26.7	24.6	<.001
Waist-hip ratio§	0.92	0.88	<.001	0.93	0.88	<.001	0.93	0.89	<.001
Total calorie intake, kcal/d§	2008.8	2151.3	<.001	2025.9	2102.8	.13	2082.3	2113.0	.14
Fat intake, % of total calorie intake§	33.7	30.6	<.001	33.8	31.8	<.001	32.8	34.6	.01
Carotenoid intake, RE/1000 kcal/d§	176.0	511.3	<.001	202.6	460.3	<.001	136.5	317.7	<.001
Alcohol intake, % current drinkers§	18	17	.87	29	23	.65	38	23	<.001
Vitamin or mineral use, %§	33	59	<.001	39	62	<.001	23	53	<.001

*Data are given as mean unless otherwise specified. RE indicates retinol equivalents. To convert cholesterol from milligrams per deciliter to millimoles per liter, multiply milligrams per deciliter by 0.02586.
†Cutoff values for quintiles of serum β-carotene were 8.8, 13.9, 20.3, and 31.1 μg/dL among never smokers; 8.5, 14.3, 20.1, and 30.3 μg/dL among ex-smokers; and 5.7, 8.4, 12.2, and 18.5 μg/dL among current smokers.
‡Trend across all 5 quintiles.
§Age, sex, and race adjusted.

Table 3. Serum β-Carotene Level by Level of C-Reactive Protein*

	Serum β-Carotene Level, μg/dL		
C-Reactive Protein Level	Never Smokers	Ex-smokers	Current Smokers
Model 1†			
Undetectable (<0.22 mg/dL)	18.9	19.0	11.6
Mildly elevated (0.22-0.99 mg/dL)	14.7	14.4	11.7
Clinically elevated (≥1.00 mg/dL)	11.0	11.7	8.3
Model 2‡			
Undetectable (<0.22 mg/dL)	18.0	18.1	11.3
Mildly elevated (0.22-0.99 mg/dL)	16.1	15.7	10.2
Clinically elevated (≥1.00 mg/dL)	13.6	13.9	9.4

*Data are given as geometric means. P<.001 for trend for all.
†Adjusted for age, race, and sex.
‡Further adjusted for systolic blood pressure, serum cholesterol, body mass index, waist-hip ratio, total energy intake, total fat intake, carotenoid intake, alcohol intake, use of vitamin or mineral supplements, use of aspirin or other nonsteroidal anti-inflammatory drugs, estrogen replacement therapy in women, diabetes mellitus, and prevalent cardiovascular disease.

Table 4. Serum β-Carotene Levels by Quintile of White Blood Cell (WBC) Count*

Quintile of WBC Count†	Serum β-Carotene Level, μg/dL		
	Never Smokers	Ex-smokers	Current Smokers
Model 1‡			
First	19.1	19.6	12.5
Second	19.7	16.7	11.9
Third	16.1	18.2	10.9
Fourth	16.5	15.9	10.2
Fifth	14.1	14.6	10.3
P for trend	<.001	<.001	<.001
Model 2§			
First	18.3	18.5	11.9
Second	18.6	16.5	11.4
Third	16.4	17.9	11.1
Fourth	17.2	16.2	10.3
Fifth	15.4	16.5	10.5
P for trend	<.001	.05	.001

*Data are given as geometric means.
†Cutoff values for WBC quintiles were 5415, 6401, 7413, and 8756/μL.
‡Adjusted for age, race, and sex.
§Further adjusted for systolic blood pressure, serum cholesterol, body mass index, waist-hip ratio, total energy intake, total fat intake, carotenoid intake, alcohol intake, use of vitamin or mineral supplements, use of aspirin or other nonsteroidal anti-inflammatory drugs, estrogen replacement therapy in women, diabetes mellitus, and prevalent cardiovascular disease.

ter adjusting for age, sex, and race, the geometric mean levels of serum β-carotene for never smokers in the lowest and highest quintiles of WBC count were 19.1 and 14.1 μg/dL, respectively (P<.001 for trend). After multivariate adjustment, the geometric mean levels for the first and fifth quintiles of WBC count were 18.3 and 15.4 μg/dL in never smokers, 18.5 and 16.5 μg/dL in ex-smokers, and 11.9 and 10.5 μg/dL in current smokers (P=.001 for trend for all).

To evaluate the possibility that the inverse association between serum β-carotene levels and markers of inflammation was due to the presence of clinical conditions that might affect β-carotene levels, inflammatory markers, or both, we repeated our analyses after excluding 3038 individuals who had prevalent diabetes mellitus or cardiovascular disease. As displayed in the **Figure**, the results were essentially unchanged.

COMMENT

In a nationally representative survey (NHANES III), we documented that serum β-carotene concentration is strongly and inversely associated with systemic markers of inflammation (CRP level and WBC count). After adjustment for carotene intake and other possible confounders, persons with elevated systemic markers of in-

©2001 American Medical Association. All rights reserved.

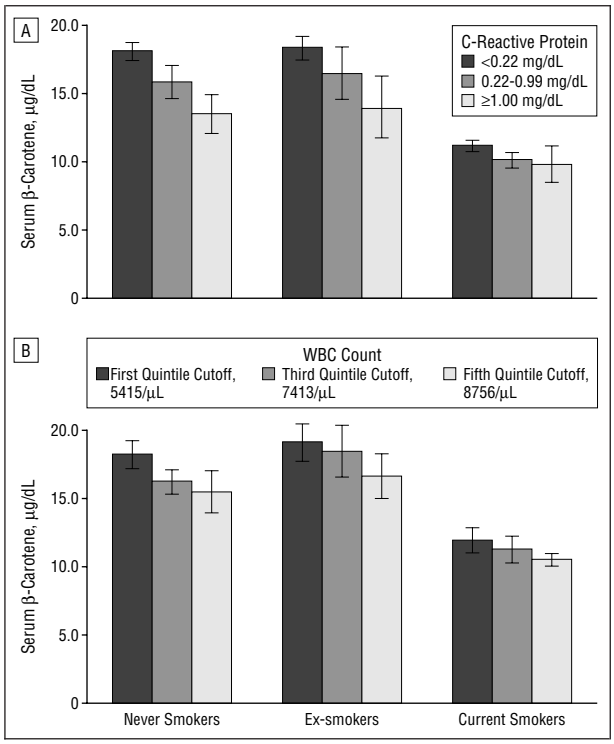
flammation had significantly lower levels of serum β-carotene. This inverse association between serum β-carotene levels and systemic markers of inflammation was demonstrated in never smokers, ex-smokers, and current smokers and persisted after exclusion of persons with clinical conditions that might confound the association.

An inverse association between serum β-carotene level and systemic markers of inflammation in a healthy population is biologically plausible. In adults with an acute illness, there is a transient decrease in serum β-carotene level with a simultaneous increase in CRP level, both of which normalize with resolution of the illness.[18,19] A similar acute-phase reaction has been shown for vitamin A and other serum vitamins and minerals.[32] In children, serum retinol levels decrease during acute infection and return to normal, without vitamin A supplementation, once the acute process has passed.[33] This pattern of findings could result from decreased production of retinol binding protein by the liver and/or increased urinary excretion of retinol during acute inflammation.[32,34] Thus, although stores of vitamin A might be depleted during acute inflammation, the decreases seem to be primarily due to the timing and magnitude of the acute-phase response. With the recent identification of a binding protein for β-carotene,[35] a similar relationship between the acute-phase response and serum β-carotene level could be hypothesized.

Although reduced serum β-carotene concentration is probably the result of systemic markers of inflammation, another interpretation of these findings is that β-carotene has anti-inflammatory properties. This conclusion is not supported by trials that show either no effect or a modest enhancement of immune system activity with supplemental β-carotene,[36-42] but additional data from clinical trials are needed to determine whether supplemental β-carotene affects systemic markers of inflammation.

Among the strengths of our analyses are the large, nationally representative survey and the remarkable consistency of our results in each category of smoking status, which persisted after adjustment for multiple potential confounders. One potential limitation is the imprecision of the measurements of CRP, WBC, serum β-carotene, and dietary intake, all based on single determinations. Still, we found highly significant associations between inflammatory markers and serum levels of β-carotene.

Results from our analyses have several implications. These findings might partially explain the discrepancy between observational studies that associated low serum β-carotene levels with increased disease risk and clinical trials of β-carotene supplements. For instance, in the Alpha-Tocopherol, Beta-Carotene trial,[6] low baseline serum levels of β-carotene were associated with an increased risk of lung cancer, whereas supplementation of the diet with β-carotene for 5 to 8 years actually increased incident lung cancer and cardiovascular disease events. One reason for these discordant results might be that low levels of serum β-carotene reflect systemic markers of inflammation, itself a risk factor for cardiovascular disease and perhaps cancer. To this end, prospective observational studies of serum β-carotene and subsequent disease risk, adjusted for inflammatory markers, would be informative, as would clinical trials that assess the effect of β-carotene supplementation on markers of inflammation.



Geometric mean levels of serum β-carotene by category of C-reactive protein (A) and the first, third, and fifth quintiles of white blood cell (WBC) count (B) adjusted for systolic blood pressure, serum cholesterol, body mass index, waist-hip ratio, total energy intake, alcohol intake, use of vitamin or mineral supplements, use of aspirin or nonsteroidal anti-inflammatory drugs, and estrogen replacement therapy and excluding persons with diabetes mellitus and prevalent cardiovascular disease. Error bars indicate 95% confidence intervals.

More broadly, our findings document the potential limitations of using serum nutrient levels as a surrogate for dietary intake, particularly in observational studies that assess the relationship between nutrient intake and subsequent disease. Serum nutrient levels have appeal in epidemiologic studies in that they are more objective and might even be more precise than corresponding estimates from a single food frequency questionnaire or multiple 24-hour dietary recalls. However, as documented in this study, physiologic processes also affect serum levels and might reduce precision. Furthermore, serum nutrient levels are still subject to confounding with other nutrients and, in fact, are subject to additional confounding from physiologic determinants.

In summary, serum β-carotene level is strongly and inversely associated with systemic markers of inflammation, which themselves are markers of increased ASCVD risk and perhaps cancer. These findings have important implications for the interpretation of studies that show an increased risk of cancer and ASCVD in persons with reduced levels of serum β-carotene. More broadly, these results highlight the potential limitations of using serum nutrient levels as a surrogate for dietary intake in observational studies. For β-carotene and likely other nutrients, it seems unwise to interpret biomarker data as prima facie evidence of dietary intake without a more com-

©2001 American Medical Association. All rights reserved.

plete understanding of the physiologic processes that affect nutrient levels.

Accepted for publication February 22, 2001.

This work was supported in part by grant T32PE10025 from the National Institutes of Health, Bethesda, Md.

Corresponding author and reprints: Thomas P. Erlinger, MD, MPH, Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins Medical Institutions, 2024 E Monument St, Suite 2-600, Baltimore, MD 21205 (e-mail: terlinge@jhmi.edu).

REFERENCES

1. Peto R, Doll R, Buckley JD, Sporn MB. Can dietary beta-carotene materially reduce human cancer rates? *Nature.* 1981;290:201-208.
2. Kritchevsky SB. β-Carotene, carotenoids and the prevention of coronary heart disease. *J Nutr.* 1999;129:5-8.
3. Street DA, Comstock GW, Salkeld RM, Schuep W, Klag MJ. Serum antioxidants and myocardial infarction: are low levels of carotenoids and alpha-tocopherol risk factors for myocardial infarction? *Circulation.* 1994;90:1154-1161.
4. Hennekens CH, Buring JE, Manson JE, et al. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med.* 1996;334:1145-1149.
5. Leppala JM, Virtamo J, Fogelholm R, et al. Controlled trial of alpha-tocopherol and beta-carotene supplements on stroke incidence and mortality in male smokers. *Arterioscler Thromb Vasc Biol.* 2000;20:230-235.
6. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med.* 1994;330:1029-1035.
7. Omenn GS, Goodman GE, Thornquist MD, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med.* 1996;334:1150-1155.
8. Lee IM, Cook NR, Manson JE, Buring JE, Hennekens CH. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: the Women's Health Study. *J Natl Cancer Inst.* 1999;91:2102-2106.
9. Cook NR, Stampfer MJ, Ma J, et al. Beta-carotene supplementation for patients with low baseline levels and decreased risks of total and prostate carcinoma. *Cancer.* 1999;86:1783-1792.
10. Albanes D, Malila N, Taylor PR, et al. Effects of supplemental alpha-tocopherol and beta-carotene on colorectal cancer: results from a controlled trial (Finland). *Cancer Causes Control.* 2000;11:197-205.
11. Das I. Raised C-reactive protein levels in serum from smokers. *Clin Chim Acta.* 1985;153:9-13.
12. Stryker WS, Kaplan LA, Stein EA, Stampfer MJ, Sober A, Willett WC. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. *Am J Epidemiol.* 1988;127:283-296.
13. Comstock GW, Menkes MS, Schober SE, Vuilleumier JP, Helsing KJ. Serum levels of retinol, beta-carotene, and alpha-tocopherol in older adults. *Am J Epidemiol.* 1988;127:114-123.
14. Boosalis MG, Snowdon DA, Tully CL, Gross MD. Acute phase response and plasma carotenoid concentrations in older women: findings from the Nun Study. *Nutrition.* 1996;12:475-478.
15. Talwar D, Ha TK, Scott HR, et al. Effect of inflammation on measures of antioxidant status in patients with non–small cell lung cancer. *Am J Clin Nutr.* 1997; 66:1283-1285.
16. Iribarren C, Folsom AR, Jacobs DR Jr, Gross MD, Eckfeldt JH. Patterns of covariation of serum beta carotene and alpha tocopherol in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Nutr Metab Cardiovasc Dis.* 1997;7:445-458.
17. Louw JA, Werbeck A, Louw ME, Kotze TJ, Cooper R, Labadarios D. Blood vitamin concentrations during the acute-phase response. *Crit Care Med.* 1992;20: 934-941.
18. Plit ML, Theron AJ, Fickl H, van Rensburg CE, Pendel S, Anderson R. Influence of antimicrobial chemotherapy and smoking status on the plasma concentrations of vitamin C, vitamin E, beta-carotene, acute phase reactants, iron and lipid peroxides in patients with pulmonary tuberculosis. *Int J Tuberc Lung Dis.* 1998; 2:590-596.
19. Curran FJ, Sattar N, Talwar D, Baxter JN, Imrie CW. Relationship of carotenoid and vitamins A and E with the acute inflammatory response in acute pancreatitis. *Br J Surg.* 2000;87:301-305.
20. Danesh J, Muir J, Wong YK, Ward M, Gallimore JR, Pepys MB. Risk factors for coronary heart disease and acute-phase proteins: a population-based study. *Eur Heart J.* 1999;20:954-959.
21. McMillan DC, Wotherspoon HA, Fearon KC, Sturgeon C, Cooke TG, McArdle CS. A prospective study of tumor recurrence and the acute-phase response after apparently curative colorectal cancer surgery. *Am J Surg.* 1995;170:319-322.
22. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *JAMA.* 1998;279:1477-1482.
23. Lindberg G, Eklund GA, Gullberg B, Rastam L. Serum sialic acid concentration and cardiovascular mortality. *BMJ.* 1991;302:143-146.
24. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation.* 1998;98:731-733.
25. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men [published correction appears in *N Engl J Med.* 1997;337:356]. *N Engl J Med.* 1997; 336:973-979.
26. Held C, Hjemdahl P, Hakan WN, et al. Inflammatory and hemostatic markers in relation to cardiovascular prognosis in patients with stable angina pectoris: results from the APSIS study. *Atherosclerosis.* 2000;148:179-188.
27. Gillum RF, Ingram DD, Makuc DM. White blood cell count, coronary heart disease, and death: the NHANES I Epidemiologic Follow-up Study. *Am Heart J.* 1993; 125:855-863.
28. Centers for Disease Control and Prevention. *Plan and Operation of the Third National Health and Nutrition Examination Survey, 1988-94.* Bethesda, Md: National Center for Health Statistics; 1994. Vital Health Statistics, No. 1(32).
29. Centers for Disease Control and Prevention. *The Third National Health and Nutrition Examination Survey (NHANES III 1988-94) Reference Manuals and Reports* [book on CD-ROM]. Bethesda, Md: National Center for Health and Statistics; 1996.
30. Driskell JA, Giraud DW, Sun J, Martin HD. Plasma concentrations of carotenoids and tocopherols in male long-term tobacco chewers, smokers and nonusers. *Int J Vitam Nutr Res.* 1996;66:203-209.
31. Shah BV, Barnwell BG, Bieler GS. *SUDAAN User's Manual, Release 7.5.* Research Triangle Park, NC: Research Triangle Institute; 1997.
32. Beisel WR. Metabolic responses of the host to infections. In: Feigin RD, Cherry JD, eds. *Textbook of Pediatric Infectious Diseases.* Philadelphia, Pa: WB Saunders Co; 1998:54-69.
33. Mitra AK, Alvarez JO, Wahed MA, Fuchs GJ, Stephensen CB. Predictors of serum retinol in children with shigellosis. *Am J Clin Nutr.* 1998;68:1088-1094.
34. Rosales FJ, Ross AC. A low molar ratio of retinol binding protein to transthyretin indicates vitamin A deficiency during inflammation: studies in rats and a posterior analysis of vitamin A–supplemented children with measles. *J Nutr.* 1998; 128:1681-1687.
35. Lakshman MR, Rao MN. Purification and characterization of cellular carotenoid-binding protein from mammalian liver. *Methods Enzymol.* 1999;299:441-456.
36. van Poppel G, Spanhaak S, Ockhuizen T. Effect of beta-carotene on immunological indexes in healthy male smokers. *Am J Clin Nutr.* 1993;57:402-407.
37. Coodley GO, Nelson HD, Loveless MO, Folk C. Beta-carotene in HIV infection. *J Acquir Immune Defic Syndr.* 1993;6:272-276.
38. Santos MS, Leka LS, Ribaya-Mercado JD, et al. Short- and long-term beta-carotene supplementation do not influence T cell–mediated immunity in healthy elderly persons. *Am J Clin Nutr.* 1997;66:917-924.
39. Santos MS, Meydani SN, Leka L, et al. Natural killer cell activity in elderly men is enhanced by beta-carotene supplementation. *Am J Clin Nutr.* 1996;64:772-777.
40. Santos MS, Gaziano JM, Leka LS, Beharka AA, Hennekens CH, Meydani SN. Beta-carotene–induced enhancement of natural killer cell activity in elderly men: an investigation of the role of cytokines. *Am J Clin Nutr.* 1998;68:164-170.
41. Hughes DA. Effects of carotenoids on human immune function. *Proc Nutr Soc.* 1999;58:713-718.
42. Hughes DA. Effects of dietary antioxidants on the immune function of middle-aged adults. *Proc Nutr Soc.* 1999;58:79-84.

©2001 American Medical Association. All rights reserved.